

A comparative study of the morphology of mammalian chordae tendineae of the mitral and tricuspid valves

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ABSTRACT

It is assumed that the human heart is almost identical to domestic mammalian species, but with limited literature to support this. One such area that has been underinvestigated is that of the subvalvular apparatus level. The authors set out to examine the morphology of the subvalvular apparatus of the mammalian atrioventricular valves through gross dissection and microscopic analysis in a small-scale pilot study. The authors examined the chordae tendineae of the mitral and tricuspid valves in sheep, pig and bovine hearts, comparing the numbers of each of these structures within and between species. It was found that the number of chordae was up to twice as many for the tricuspid valve compared with the mitral valve. The counts for the chordae on the three valve leaflets of the tricuspid valve, as well as the two mitral valve leaflets, were almost identical between species. However, the chordae attaching onto the posterior papillary muscle were almost double compared with the septal and anterior papillary muscles. Histological analysis demonstrated an abrupt transitional zone. In conclusion, the authors have shown that there is no gross morphological difference between, or within, these species at the subvalvular apparatus level.

INTRODUCTION

The anatomy of the heart of domestic mammals is similar, if not identical, to humans (Colville and Bassert 2009). Specifically, the anatomy of the heart of the pig (Douglas 1972, Hughes 1986, Cooper and others 1991, White and Wallwork 1993), cow (Budras and Habel 2003) and sheep (Iaizzo 2009) share the same basic arrangement of the cardiac chambers. Indeed, in veterinary studies, the model of circulation established in humans is taught as applied to animals as there are no clinically significant differences.

Despite the fact that the anatomy of the heart in these species is generally accepted to be similar to humans, only limited anatomical studies exist within the literature.

Comparative anatomical studies in cardiac anatomy are limited, more so in the field of the atrioventricular valves (AV) of these species. Despite limited anatomical information being available about the AV valves, one area that has received attention is the application of the AV of the sheep in animal models of valvular heart disease (Ali and others 1996, Kunzelman and others 1999, Leroux and others 2012). Similarly, the pig AV valves have also been examined in relation to experimental prolapse (Quill and others 2011), and surgical correction of regurgitation affecting the mitral valve in animals (Goetzenich and others 2010). However, studies of bovine heart valves are not so apparent, with the research focusing on the use of bovine pericardium in human cardiac surgery (Vrandecic and others 1998, Chambers and others 2008). With this in mind, and the claims that anatomy of domestic mammals is similar, the authors set out to investigate the anatomy of the AV.

These valves differ to the semi-lunar valves as they have a subvalvular apparatus. Each valve leaflet is interconnected with string-like chordae tendineae that anchor the leaflets to prevent the valves inverting into the atria under pressure. This anchoring system is even more effective due to the continuous collagen sheet running from the ring-like annulus into the valve cusps and through the chordae tendineae (Fenglio and others 1972). The composition of chordae tendineae is well suited to the repetitive strain that they undergo during the cyclic motion of systole and diastole. Chordae are composed of avascular connective tissue with an outer sheath of elastic fibres and a dense collagen core allowing for a high level of durability; they are covered by endocardium (Millington-Sanders and others 1998). The chordae attaching to the anterior leaflet of the mitral valve have been studied more than those attaching to the posterior leaflet;



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research has shown that there are two subsets—primary and secondary. Primary chordae are thinner and attach to the free edge of the valve leaflet and potentially protect the leading edge from compressive forces during coaptation. Whereas secondary or strut chordae attach to the inferior belly of the anterior leaflet. Research has stipulated that their main function is in regards to load bearing and that they help to relieve the high systolic stress placed on the belly of the mitral leaflet (Nielsen and others 2003).

There are few comparative anatomical studies of mammalian heart tissue especially at the subvalvular level; this study aimed to provide evidence for this.

MATERIALS AND METHODS

Initial wet dissection work took place at the University of Glasgow's School of Veterinary Medicine. Nine mammalian hearts were dissected; four sheep (*Ovis aries*), three bovine (*Bos primigenius*) and two pig hearts (*Sus domestica*). This is a small-scale study due to the limited availability of the materials available from the abattoir for a study conducted as part of a small-scale Honours degree-related project. All specimens were collected from the Wishaw Abattoir (Glasgow, UK) on the day of kill and were dissected within one week. The hearts received were cut from the great vessels and pericardium, but had no prior incisions to the main body of the heart, which meant the chambers and AV valves were fully intact. All specimens were kept fresh in sealed containers within a chilled cabinet; no chemical fixatives were injected.

Using dissecting scissors, an incision was made on the left side of the heart in order to fully display the mitral valve and minimise damage to the subvalvular apparatus (Lam and others 1970). A second incision was made on the right side. Both incisions began at the entrance to the atria and descended inferiorly through the AV valves to the apices of the ventricles. The right ventricle was found to be larger than the left and required a further (third) incision that ascended superiorly and posteriorly from the ventricular apex to fully display the tricuspid valve.

In this investigation, all chordae that attached directly to the papillary muscles with a discrete point of origin were counted. Due to the range of heart sizes, this simple blanket classification method was used and applied to all specimens.

Counts were separated first by valves—tricuspid or mitral and then by papillary muscle origin—anterior or posterior in the case of the mitral valve and anterior, septal and posterior for the tricuspid valve.

Histology

Histological samples were taken from one of each of the three species where a large ribbon of tissue was cut beginning at the valve cusp and ending midway along the papillary muscle. A representative block was

examined from one of each species. Therefore, samples were taken from one sheep, one pig and one set from a cow as representative samples from the species dissected.

Each section was fixed in an individual *CellStor Pot* (CellPath, Newtown, Powys, UK) and pinned to cardboard to retain its length; each sample was then fixed for four days. Once cut by a microtome and mounted onto a slide, the tissue sections were stained for histological examination. H&E, and haematoxylin and Van Gieson stains were used on each block. Further details are provided in online supplementary appendix A. H&E stained nuclei purple and connective tissue varying shades of pink. Haematoxylin and Van Gieson stained muscle yellow and collagen bright pink/red. Staining protocols are detailed in online supplementary appendix 1. Once stained, the slides were photographed under a light microscope to document the tissue composition of the junction between the chordae and the papillary muscle.

Chordae counts were grouped into subsets according to the papillary muscle they originated from. Comparisons were made between papillary muscle counts across the species, and between mitral and tricuspid chordae counts across the species.

Statistical analysis

The data and sample groups were collected as follows. In each of the three species, chordae tendineae that were attached to each of the three valve leaflets (anterior, septal and posterior) of the tricuspid valve were counted separately and chordae that were attached to the posterior and anterior leaflets of the mitral valve were also counted separately.

These groups were then analysed and compared to gain a better statistical understanding.

For the counts of the chordae that were attached to the tricuspid valve leaflets median, minimum and maximum values were calculated. The same calculations were applied to the chordae that were attached to the mitral valve leaflets.

The counts for the mitral and tricuspid valve leaflet were then compared and averaged within each species. Table 1 illustrates the average counts in each of the three species along with the P value and the total counts for each valve. Tables 2 and 3 highlight the median,

TABLE 1: Mean chordae count for atrioventricular valve valves

Species type	Mitral	Tricuspid	P value
Sheep	12	22	0.04
Pig	16	23.3	
Bovine	20	25	
Mean (sd)	16 (4)	23.4 (1.5)	

This table highlights the mean chordae tendineae counts across four species for each of the mitral and tricuspid valves, and related P values

TABLE 2: Chordae counts for the mitral valve

Species type	Number of specimens in each species	Minimum	Median	Maximum
Sheep	4	11	15	20
Pig	2	17	20	23
Bovine	3	12	17	22

This table displays the number of specimens dissected from each species and their respective minimum, median and maximum chordae counts for the mitral valve

minimum and maximum counts for the mitral and tricuspid valves, respectively.

Statistical analyses were applied to the data using StatsPlus in Microsoft Excel for Mac 2011 to establish whether the differences were significant. Comparing the two papillary muscle counts for the mitral valve across the species involved two groups; therefore, a Student's t test was used to calculate the P value. When comparing the three groups of counts from the papillary muscles associated with the tricuspid valve, an analysis of variance single-factor test was used to calculate the P value.

RESULTS

Gross dissection

Results were obtained from the examination of nine hearts in total—four sheep, two pig and three bovine hearts. At a gross anatomical level, it would appear that the surface morphology at the junction with the papillary muscle and the chordae is consistent across the given species (Fig 1). This shows similarities of the subvalvular apparatus of the mitral valve in bovine, pig and sheep hearts in terms of origin, distribution and termination in the valve leaflets. Tables 2 and 3 highlight the counts of the mitral and tricuspid valve leaflets across the three species used.

Figure 2 demonstrates the numbers of the chordae within the sheep examined (n=4) of each of the anterior (8), septal (8) and the posterior papillary muscles (6)

TABLE 3: Chordae counts for the tricuspid valve

Species type	Number of specimens in each species	Minimum	Median	Maximum
Sheep	4	18	23	28
Pig	2	23	24	26
Bovine	3	21	25	28

This table displays the number of specimens dissected from each species and their respective minimum, median and maximum chordae counts for the tricuspid valve

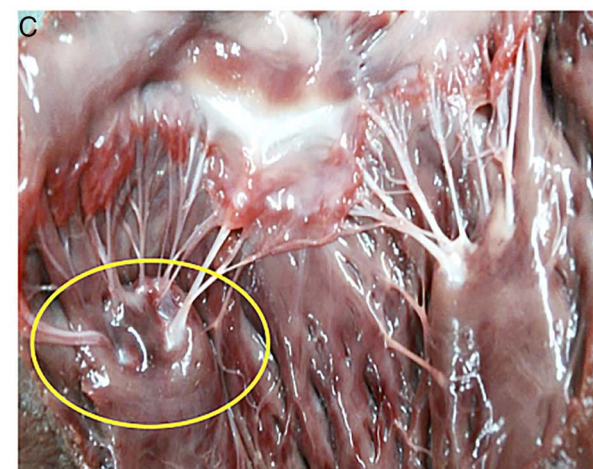
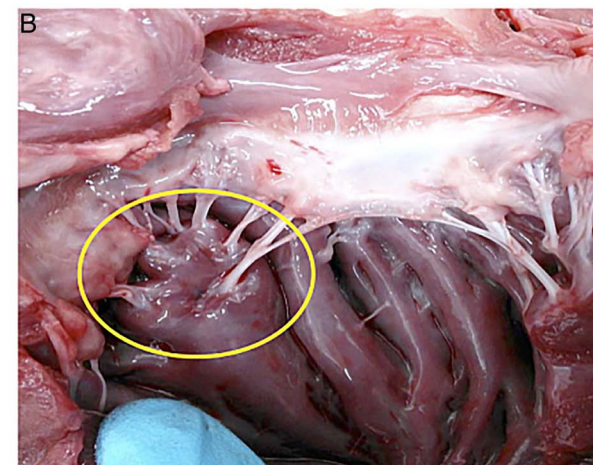
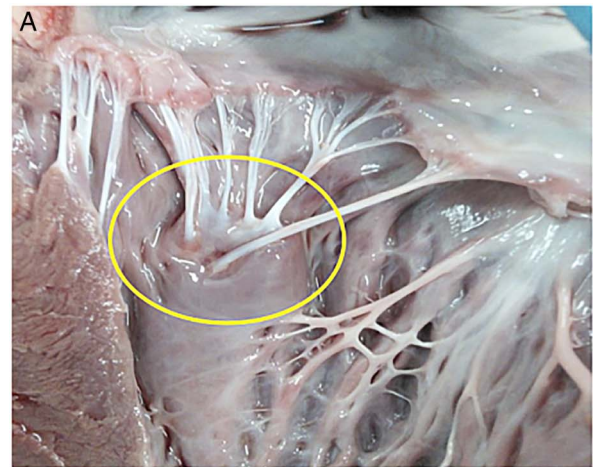


FIG 1: Subvalvular apparatus of the mitral valve (a, bovine; b, pig; c, sheep). At the top of each image, the valve leaflets of the mitral valve can be seen. Below the cusps are the interconnecting chordae that descend to anchor into the papillary muscles. On the left of each photograph, the chordae anchor into the apical portion of the anterior papillary muscle (circled). From observation, it can be seen that the surface morphology of the apical surface is similar across the given species

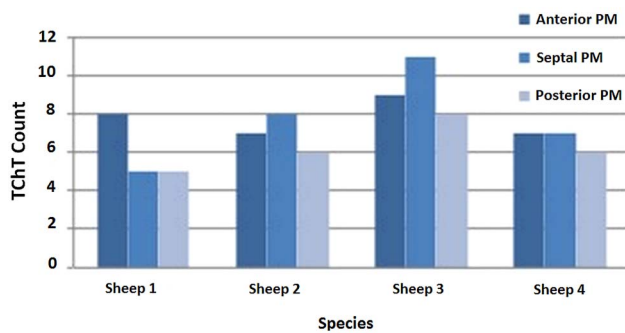


FIG 2: Chordae counts for papillary muscles (PM) of the tricuspid valve in the sheep. The tricuspid valve has three leaflets: anterior, septal and posterior

for the tricuspid valve. No significant difference between each of the papillary muscles was identified within the same species (sheep), where $p=0.40$. Across the three species examined (sheep, bovine and pig), the numbers of chordae for each of the three papillary muscles were similar for the anterior and septal papillary muscles, but with slightly more chordae tendineae for the posterior papillary muscle (12) in the pig (Fig 3).

In relation to the chordae counts for the mitral valve, there was half the number of chordae tendineae for each of the two leaflets compared with the tricuspid valve in the sheep and bovine specimens. In the pig, the difference was not as marked, with the mitral valve having 25% less chordae tendineae compared with the tricuspid valve (Fig 4).

Histological analysis

The valve leaflets and the chordae are both composed of collagen, and therefore at this transition point, there was no distinguishable change in the tissue. Sampling for histology involved taking sections from the junctional region between the chordae and the papillary muscle.

Due to the differences in scaling, there can be no quantitative comparison of chordae collagen core density. However, the characteristics of the junction

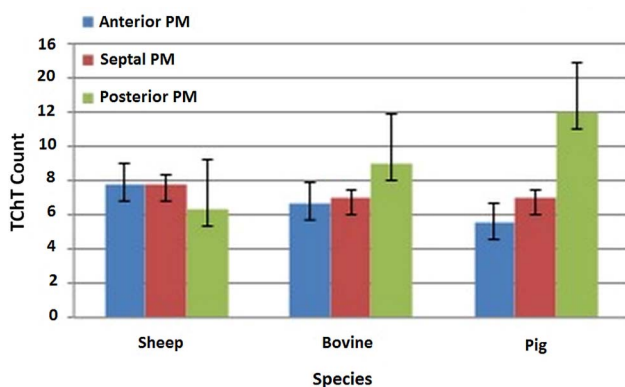


FIG 3: Comparison of the chordae counts of each of the three valve leaflets, comparing across the three species examined. PM, papillary muscles

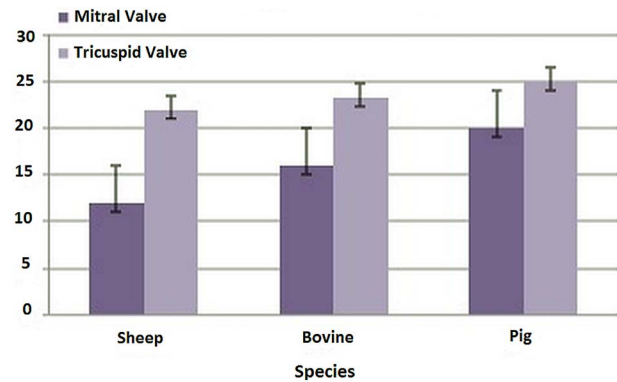


FIG 4: Comparison of total chordae counts for mitral and tricuspid valves across the given species. There is a significant difference between the counts of chordae in the mitral and tricuspid valves, with a significant P value of 0.04 in Table 1. This shows there are more chordae in the tricuspid valve compared with the mitral valve

between the collagen of the chordae and the muscle fibres of the papillary muscles can be determined. These observations showed that the transition from one tissue type to the other is relatively abrupt in the three species (Fig 5).

An intraspecies comparison can be made with pig sub-valvular apparatus from the left and right sides of the heart, as shown in Fig 6. From the histological slides, the bright pink collagen core of the mitral chordae is denser than that from the tricuspid valve, although this was not quantitatively assessed. In addition, the fibrous component of the chordae extended into the fibrous arrangement of the valve leaflets in both mitral and tricuspid valves in all three species (not shown).

DISCUSSION

The authors have shown that the gross morphology of the chordae attaching at the valve leaflet and the papillary muscle was very similar, if not identical in arrangement, both within the species, and compared across the three species. It has been shown that in the human heart there are 8–12 chordae tendineae in each of the mitral valve leaflets (Millington-Sanders and others 1998). In this study, the authors have shown, on average, for this to be similar in sheep (12), cow (16) and pig (20) for the total count for both valve leaflets combined. As there are two valve leaflets, and as Millington-Sanders and others (1998) indicated that there will be approximately 16–24 in total for both mitral valve leaflets combined, these results are almost identical across the species examined in this study. This adds further to the literature as the anatomy of the mitral valve has only been examined in pigs in terms of the base width at point of insertion in the papillary muscle, cusp length and ring circumference, but it was not clearly defined in relation to the chordae tendineae by Lima et al. what they were examining in this respect (Lima and others

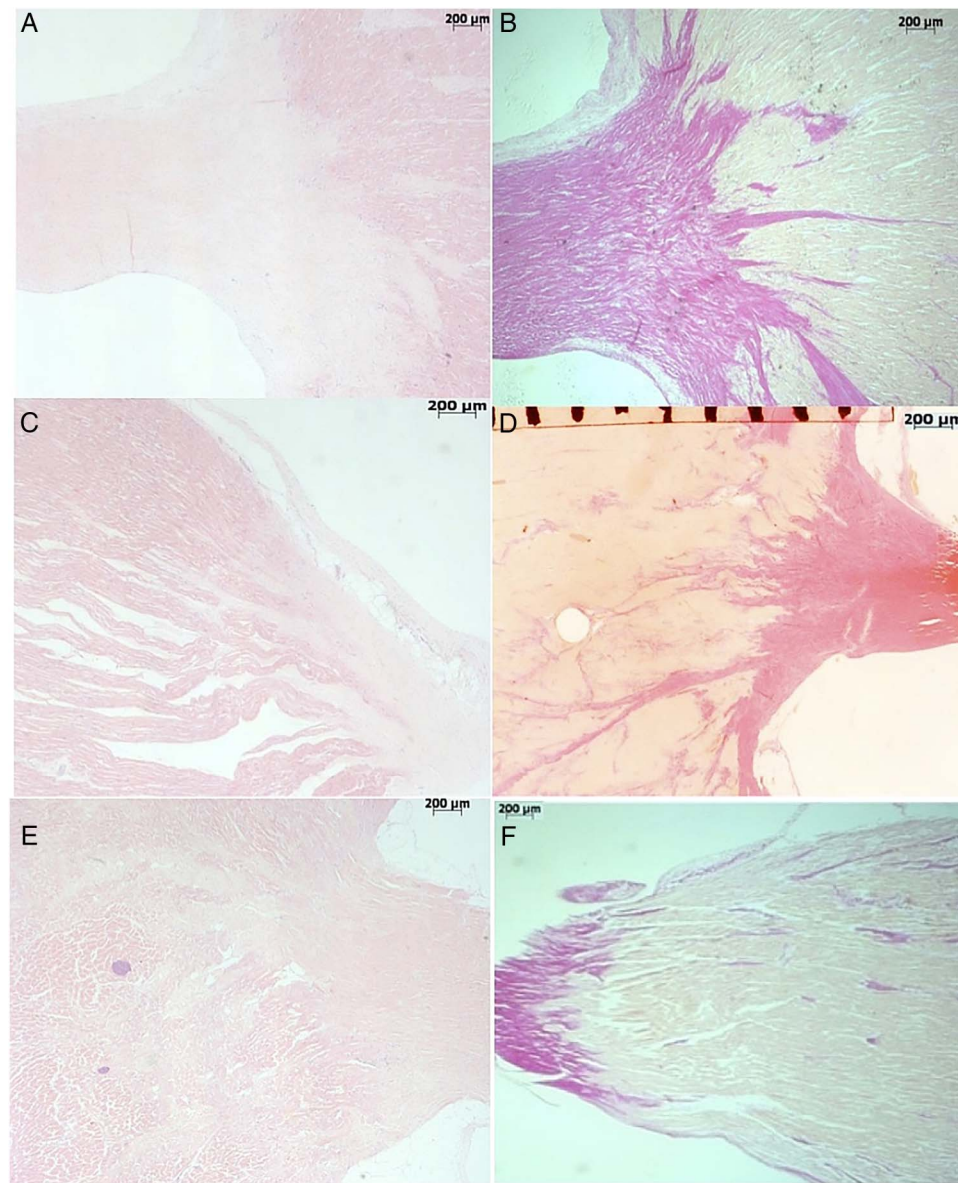


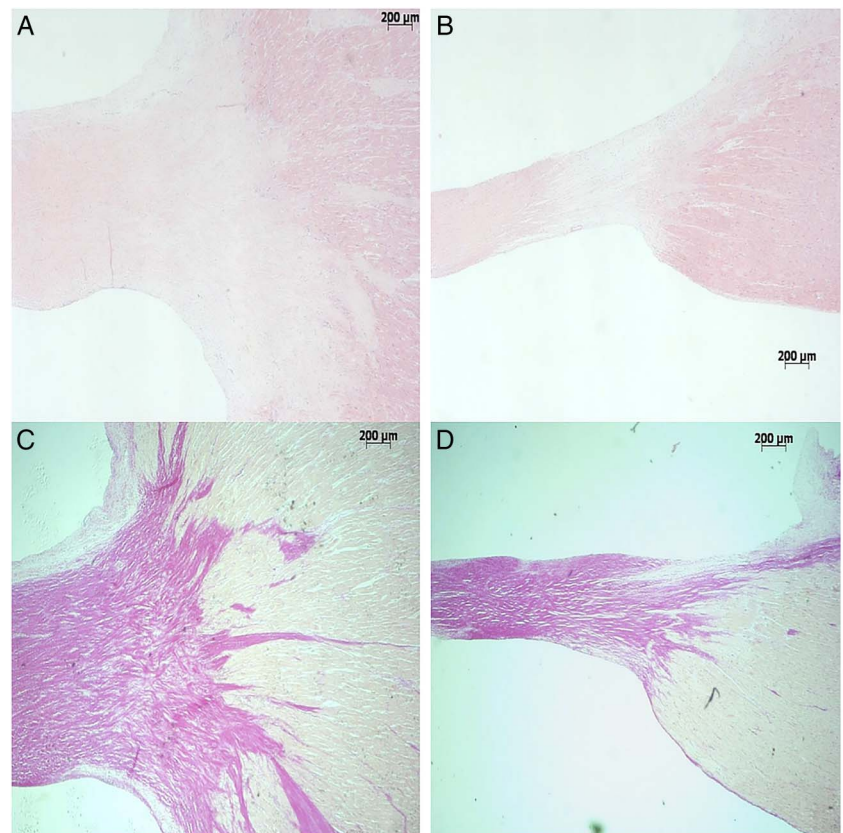
FIG 5: Histological sections from the transition zone of a chordae tendineae anchoring into an anterior papillary muscle of the mitral valve. (a) and (b) Pig. (c) and (d) Bovine. (e) and (f) Sheep. (a), (c) and (e) H&E stain: junction noticeable but not distinct. (b), (d) and (f) Haematoxylin and Van Gieson stain: papillary muscle stained yellow and the collagen core of the chordae is stained bright pink. To fully view the tissue transition in the bovine chordae (d), a larger magnification was used. Each bar represents 1 mm on this section

2013). There is only limited information about the sub-valvular apparatus, and most of the literature focuses on the use of the mitral valve in pigs from the perspective of bioprosthetic material in xenografts (Cohn and others 1981, Pavoni and others 2007), stent implantation (Attmann and others 2011) or abnormal physiological function (Quill and others 2011).

On examining the literature in the field of the mitral valve's chordae tendineae of sheep, most of the (limited) studies examine the histological components of collagen composition (Berkovitz and Robinson 1991) or from the physiological perspective, or replacement of sheep valves with prosthetic components (Vetter and others 1986, Nielsen and others 2003). However, the

single study the authors were able to examine in relation to the chordal structure counts in sheep mitral valve revealed similar results to this study. A similar study found that there were 10–27 chordae tendineae in the sheep's mitral valve, which fits closely to the work presented here (average of 12 chordae), though the total counts were on the lower end of what they found (Ferreira de Queirox and others 2009). A comparative study examining the human heart and sheep chordae tendineae in the mitral valve stated that there was a statistically significant difference in numbers of chordae tendineae between sheep and humans (Ozbag and others 2005). In relation to bovine chordae tendineae, there were no obvious data in the literature examining

FIG 6: Histological sections of chordae tendineae from a pig's heart. This figure displays samples dissected and stained from the same pig heart. (a)–(d) all show a mid-section from a chordae inserting into an anterior papillary muscle. (a) and (c) are taken from the mitral valve, (b) and (d) from the tricuspid valve. (a) and (b) are stained with H&E, and illustrate the transition from the chordae's collagenous core to the muscle of the papillary muscle. (c) and (d) are stained with haematoxylin and Van Gieson, which discretely demonstrates the junction between the pink stained collagen and yellow stained muscle



the mitral valve's chordae. The authors found that, on average, there were 16 chordae for the mitral valve combining both valve leaflets. Instead, the literature tends to focus on the use of bovine pericardium in the replacement of valves in humans ([Vrandecic and others 1998](#), [Pomerantzeff and others 2005](#), [Li and others 2011](#)). For the pig, again, data were not apparent on the chordae counts for the mitral valve, but the authors have shown that they have slightly more than the sheep and cow, with an average total of 20 chordae.

With respect to the tricuspid valve and the attachments of the chordae to each of the valve leaflets, there is limited material in the literature in this field. In one study, it was found that the number of chordae tendineae in sheep, compared with humans, was very similar ([Motabagani 2006](#)). Indeed, from the perspective of the pig, only basic anatomy of this site is available ([Donnersberger and Scott 2005](#)). Also for the cow, only detailed studies tend to exist reporting the use of the pericardium ([Vrandecic and others 1998](#), [Chambers and others 2008](#)), rather than detail about this valve, and related apparatus. Although studies of the human tricuspid valve and related chordae tendineae exist, and focus on the morphology of these structures ([Lam and others 1970](#), [Silver and others 1971](#)), comparative anatomical studies are not apparent.

The authors have provided evidence, as far as they are aware for the first time, comparing the number of chordae tendineae of the tricuspid and mitral valves. The authors have shown, as may be expected, that there

are significantly more chordae tendineae in the tricuspid valves of bovine, sheep and pig samples. Indeed, the authors have also shown that the average numbers of total chordae tendineae of the tricuspid valve are very similar across the species (22 sheep; 23 bovine and 25 pig). This compared with 12, 16 and 20 chordae for the sheep's, cow's and pig's mitral valves, respectively.

The other focus of this study was in the histological appearance at the junction of the chordae and the mitral and tricuspid valves at the papillary muscle end. One comparative study used the scanning electron microscope comparing human and swine chordae tendineae from the left ventricle (mitral valve) ([Gusukuma and others 2004](#)). They showed that the deep layer of the chordae tendineae under the lining endothelium was comprised of oblique and longitudinal collagen fibres. The major difference noted between swine and human samples was that in the pig the fibres were orientated at random, whereas in the human samples the collagen fibres were structured more uniformly.

In this study, the authors showed that while the arrangement of the collagen fibres in the chordae tendineae were uniform in their arrangement, the junction between the chordae and the papillary muscle was relatively abrupt at the junctional region. This was true not just for the pig but also applied to the cow and sheep samples examined here. This pattern of uniformity of the arrangement of the collagen fibres fits with the arrangement of other tendons, where the collagen fibres are orientated along the direction of loading ([Fung 1981](#)).

However, studies in the rabbit and sheep, goat and human chordal structures have shown similar results as to the orientation of the collagen fibres, as the authors have also shown. This is surprising as the chordae are placed under a great amount of tension, and therefore, further penetration of collagen fibres into the papillary muscle would improve their anchorage. In the literature, the reasoning given for this is the presence of microfibrils in the muscle fibres of the basal lamina of the papillary muscle that securely anchor the collagen fibres [30]. Future research to fully analyse this tissue transition would require quantitative measurements to examine the exact depth of penetration of the collagen fibres into the papillary muscle. Again there were no obvious interspecies differences in the transition areas.

The main differentiating feature that the authors have demonstrated, as the authors believe for the first time, is the transitional zone's relatively abrupt change at the junction of the chordae with the papillary muscles.

Limitations of the study

Within this small-scale study, one limitation was in the numbers used. Due to limited availability of specimens from the abattoir and a short timeframe of this project, the authors were only able to gain access to nine specimens in total (four sheep, three bovine and two pig hearts). For future work, increasing the numbers of specimens analysed can expand this. In addition, this study has not examined the difference between primary and secondary mitral cords, which are important in the dynamics of the left ventricle.

However, against this limitation, the authors have enhanced the understanding of numbers of chordae attaching to the valve leaflets of the mitral and tricuspid valves. In addition, they have also demonstrated that the histological appearance of the chordae at the junction with the papillary muscle has similarities and differences to other studies. The other advantage of this study is based in the fact that the authors also did a comparative anatomical study highlighting that the similarities to human heart valves are almost identical to that of the sheep, cow and pig.

CONCLUSION

To the authors' knowledge, this is the first study to report on the comparative anatomy of the subvalvular apparatus of the heart in sheep, cow and pig. They have shown that there is significantly more chordae tendineae attaching onto the tricuspid valve leaflets than the mitral valve. In addition, they have shown that the relative numbers of chordae tendineae attaching onto the mitral valve across the species examined, and also for the tricuspid valves, were very similar. The only difference they have highlighted is that there are more chordae attached onto the posterior papillary muscle compared with the anterior and septal papillary muscles of the tricuspid valve leaflets only in the pig. Also, they have

demonstrated a clear and relatively abrupt transition zone between the chordae and the papillary muscles, and this may have a functional implication, but this needs to be investigated further.

This study enhances our understanding of interspecies and intraspecies anatomy of an underinvestigated area of animal cardiac anatomy. This will inform the veterinary clinician and add further weight to the use of animal heart tissue to teach human anatomy and reflects the similarities between these species and man.

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Contributors JH was involved in carrying out the tissue analysis, dissection and histology processing as well as construction of the manuscript. PMR was involved in the concept and study design, analysis, interpretation and construction of the manuscript.

Competing interests None declared.

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Data sharing statement We believe in open access as much as possible to the data set in this study.

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